PROTOCOL · RPMOTION

Organoid culture in the RPMotion

ORGANOID TYPE — LIVER, PANCREAS, SMALL INTESTINE AND KIDNEY

EXPANSION & DIFFERENTIATION

AT A GLANCE

4
vessel positions per module, each set independently

40–100
RPM run in parallel when optimising a new organoid type

14 d
expansion, 0.5M cells inoculated to 20M at harvest

9 d
differentiation, transferred straight after expansion

General description

The RPMotion is a bioreactor designed for organoid culture in suspension. Motor-driven stirring keeps cultures in motion at a controlled speed. Each module accommodates four culture vessels, with independently adjustable stirring speeds for each position.

Device and control

Each culture vessel consists of a standard conical centrifuge tube, a reusable stirring rotor supplied by Orgonex and a proprietary single-use filter lid. The vessels are placed in the RPMotion module, which connects to a touchscreen Control Unit. Stirring speeds can be set individually for each vessel or matched across all four positions.

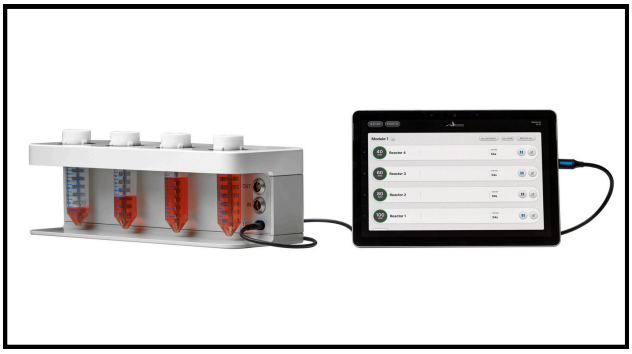


FIG. 1 — RPMOTION MODULE, WITH FOUR CULTURE VESSEL POSITIONS AND CONTROL UNIT

Materials

Dynamic organoid culture in the RPMotion uses standard laboratory equipment and consumables. The media and solutions used for static culture can also be used for dynamic culture, with volumes adjusted to the culture conditions.

CELL COUNTER

Determine the cell number before inoculation to establish the required seeding density.

CENTRIFUGE

Standard benchtop centrifuge compatible with conical centrifuge tubes

PIPETTES

Serological pipettes and micropipettes for medium changes and liquid handling.

AUTOCLAVE

For sterilising reusable stirring rods after cleaning

MEDIA & SOLUTIONS

Appropriate expansion or differentiation media and solutions for the organoid type.

VESSEL PARTS

A compatible conical centrifuge tube, a reusable Orgonex stainless steel stirring rod and a single-use filter lid.

Safety & handling

Sterility

Assemble culture vessels and perform medium changes in a biological safety cabinet using aseptic technique. Keep filter lids dry and minimise the time vessels remain open.

Device handling

Place the RPMotion module on a stable, level surface inside the incubator. Route cables through the rear access port or past the rubber door seal, avoiding pinching or strain and ensuring the door closes and seals properly. Operate within the limits specified in the Orgonex instructions for use.

Cleaning & sterilisation

Clean and autoclave reusable stirring rods according to the Orgonex instructions for use, using the specified autoclave cycle. Do not autoclave the RPMotion device, culture tubes or filter lids.

Biosafety

Follow institutional biosafety procedures appropriate to the cells and materials used. Dispose of cultures, media and single-use components through the designated biological waste streams.

Procedure – how to use the RPMotion

01 **Prepare the components –** Clean and autoclave the reusable stirring rods according to the Orgonex instructions for use. Filter lids are supplied sterile and ready to use. Do not autoclave the RPMotion device.

02 **Set up the device and select the stirring speed** - Place the RPMotion module inside the incubator and connect it to the Control Unit. Route cables through the rear access port or past the rubber door seal, ensuring the door closes and seals properly. Set the stirring speed for each vessel according to the organoid type and culture phase.

Organoid type	Seeding density	Expansion (rpm)	Differentiation (rpm)
Liver organoids (ICO)	1x10^5 cells/mL	60-80	100
Pancreatic organoids	1x10^5 cells/mL	100	Not determined
Small intestinal organoids	0.5x10^5 cells/mL	100	80
Kidney organoids	0.5x10^5 cells/mL	80	80

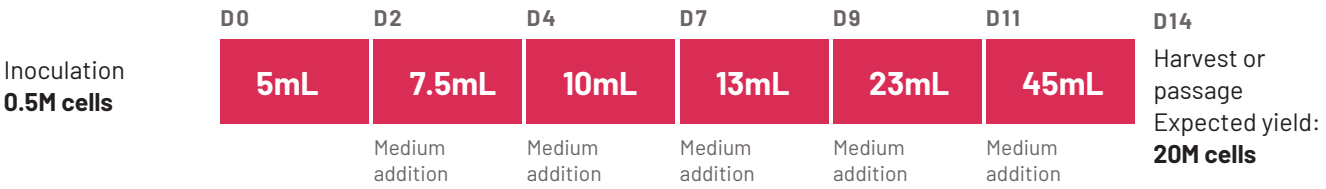
Optimisation – For a new organoid type, compare 40, 60, 80 and 100 rpm in parallel to identify suitable stirring conditions.

03 **Prepare the inoculum** - Dissociate organoids from static culture using the appropriate dissociation method for the organoid type.

04 **Determine the seeding density** - Count the cells before inoculation and calculate the number required per vessel. Use the seeding density specified for the organoid type.

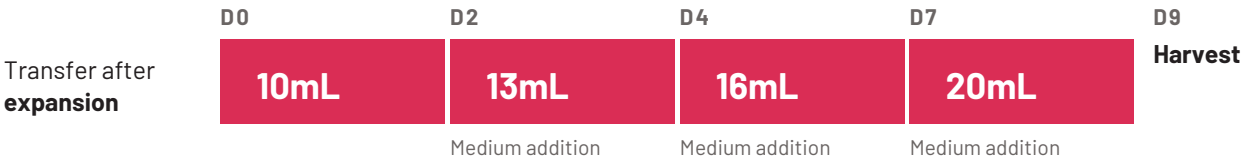
05

Organoid expansion - Inoculate the vessels and add expansion medium according to the schedule below. The volumes shown represent the total culture volume at each time point.



06

Organoid differentiation - Following expansion, transfer the organoids to differentiation medium and adjust the stirring speed according to the table above. Add differentiation medium according to the schedule below. The volumes shown represent the total culture volume at each time point. Example below for liver organoids (hepatic differentiation).



Culture in progress

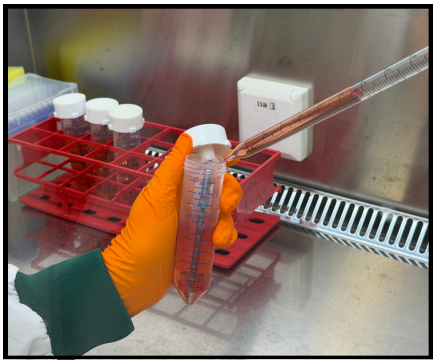


FIG. 2 — ADDITION OF CULTURE MEDIUM TO THE VESSEL USING A SEROLOGICAL PIPETTE.



FIG. 3 — CULTURE VESSELS POSITIONED IN THE RPMOTION MODULE FOR ORGANOID EXPANSION OR DIFFERENTIATION.

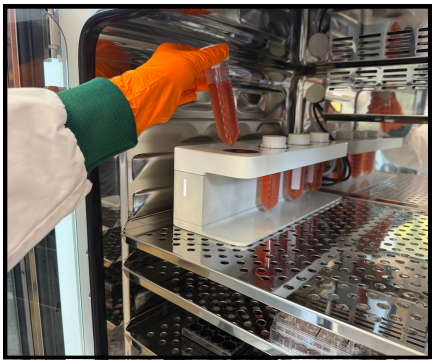


FIG. 4 — REMOVAL OF A CULTURE VESSEL FROM THE RPMOTION MODULE FOR HARVESTING OR PASSAGING.

Questions about this protocol?

Orgonex develops bioreactor technology for organoid culture. For advice on seeding densities, stirring speeds or adapting this protocol to your organoid type, contact us at orgonex.com/contact.